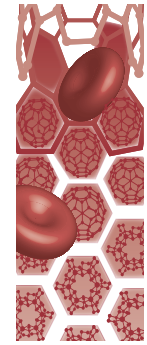
Review

Nanomedicine



Antitumor immunity by magnetic nanoparticle-mediated hyperthermia

Magnetic nanoparticle-mediated hyperthermia (MNHT) generates heat to a local tumor tissue of above 43°C without damaging surrounding normal tissues. By applying MNHT, a significant amount of heat-shock proteins is expressed within and around the tumor tissues, inducing tumor-specific immune responses. *In vivo* experiments have indicated that MNHT can induce the regression of not only a local tumor tissue exposed to heat, but also distant metastatic tumors unexposed to heat. In this article, we introduce recent progress in the application of MNHT for antitumor treatments and summarize the mechanisms and processes of its biological effects during antitumor induction by MNHT. Several clinical trials have been conducted indicating that the MNHT system may add a promising and novel approach to antitumor therapy.

Keywords: antitumor therapy • heat-shock proteins • hyperthermia • immune response • magnetic nanoparticles • tumor immunity

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Hyperthermia provides a physical treatment that results in thermoimmunotherapy with significantly fewer side effects than in chemotherapy or radiotherapy, and may add promising and novel approaches to antitumor therapy. However, the difficulty of heating a local tumor tissue to the desired temperature without damaging surrounding normal tissues is a major technical problem in the currently available hyperthermia modalities [1,2]. A consensus landmark is the threshold temperature of 43°C [3]. Above this threshold temperature, heat cytotoxicity is markedly higher. Hyperthermia is able to induce both necrosis and apoptosis, depending on the temperature. Susceptibility to apoptosis seems important below the consensus temperature, whereas necrosis seems more likely above it [4]. A high temperature of above 43°C can kill a significant number of tumor cells [3], but normal tissues are also severely damaged under the conventional hyperthermia treatments, such as local mild hyperthermia (LMHT) and whole-body hyperthermia (WBHT). By contrast, magnetic nanoparticle-mediated hyperthermia (MNHT) appears to overcome these shortcomings [5–7]. MNHT treatment consists of two major stepwise technical approaches: the first is to accumulate magnetic nanoparticles (MNPs) into the tumor tissue, and the second is to apply an alternating magnetic field (AMF) in order to generate local heat through nanoparticles. By applying MNHT, the temperature of the tumor tissue is increased uniformly to above 43°C, and a significant amount of heat-shock proteins (HSPs) is expressed within and around the tumor tissue. *In vivo* experiments have indicated that MNHT can induce the regression of not only a local tumor tissue exposed to heat, but also distant metastatic tumors unexposed to heat [8]. This article briefly describes recent progress in the preparation of MNPs for MNHT and emphasizes the importance of the immune response as the mechanism and process underlying the induction of tumor-specific effects by MNHT.

MNPs suitable for MNHT

The heating power of MNPs has been reviewed by Hergt *et al.* [9] and Dennis and

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Ivkov [10], and they showed that heat is generated by Néel or Brown relaxation and hysteresis loss through AMF exposure. The preparation methods of MNPs (typically less than 100 nm in diameter) that are suitable for MNHT have been reviewed [11–14]. Any submicron MNPs that generate heat by exposure to an AMF can theoretically be used for developing MNHT. However, the most important criterion for this approach is that the MNPs should be nontoxic. Because of this requirement, magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) particles have largely been used.

Table 1 shows a list of representative MNPs that have been developed for MNHT. The history for the use of MNPs for the development of hyperthermia treatment started in 1957, when Gilchrist *et al.* first used MNPs of a few micrometers in size in order to induce local heating to lymph nodes in dogs [15]. In 1979, Gordon *et al.* used a dextran-magnetite solution with a core size of 6 nm [16]. Liposomal coatings have been used as a promising approach for MNHT treatment to tumor tissues, and magnetite cationic liposomes (MCLs) were developed in order to improve accumulation in tumor tissues [17,18]. The efficacy of MNHT using MCLs has been examined and reviewed in animal experiments using several tumor types [7,19]. Cheraghipour *et al.* also developed cationic albumin-conjugated MNPs with stable MNP suspensions and improved their adsorption to cells [20].

Another approach for the better application of MNHT may be to selectively accumulate MNPs into tumor tissues by utilizing the biological and biochemical processes that are uniquely expressed in tumor cells. For example, malignant melanoma carries out the biosynthesis of melanin pigments in the presence of a melanin-forming enzyme, tyrosinase. A tyrosine homolog, N-propionyl-cysteaminylphenol (NPr-CAP), which is a tyrosinase substrate, is conjugated directly on the surface of MNPs or indirectly through the insertion of PEG onto the surface of dextran-coated MNPs [21–23]. In several B16 mouse melanoma models, these conjugates were found to accumulate selectively in melanoma tumors.

MNPs were conjugated with monoclonal antibodies (mAbs) against human breast cancer cells [24], human mAbs against renal cell carcinoma [25], humanized mAbs against HER2 [26] or its aptamer [27] and mAbs against EGFR [28]. These were expected to provide a novel delivery and targeting approach to tumor cells. Given systemically, such nanoparticles could reach and bind specifically to tumor cells in animal models [24–25,28]. Yang *et al.* reviewed recent advances in the development of such targeted nanoparticles and discussed their broad application in drug delivery, targeted therapy, molecular imaging and the therapeutic decision-making regarding and monitoring of cancer cells [31].

Yatvin *et al.* [29] and Ponce *et al.* [30] developed thermosensitive liposomes with enwrapped MNPs and anti-ancer drugs. Local heating of the target area to a temperature of 42–44°C would cause the liposome lipids to 'melt', and the liposomes flowing through the vascular bed of a heated area would rapidly release the entrapped drug into the surrounding medium.

Table 1. Representative magnetic nanoparticles for magnetic nanoparticle-mediated hyperthermia.

Name	Core size	Characteristics	Ref.
Magnetite	A few μm	The first demonstration using magnetic particles	[15]
Dextran magnetite	6 nm	The first demonstration using magnetite of nanometer sizes	[16]
Aminosilane-coated magnetite	15 nm	Enhances the uptake by cancer cells and prevents intracellular digestion	[5]
Magnetite cationic liposome or cationic protein	10 nm	Enhances the uptake by cancer cells and stabilizes the colloidal solution	[17–18,20]
NPrCAP-conjugated magnetic nanoparticle	10 nm	Targets melanoma cells and exerts chemotherapeutic effects	[21–23]
Antibody-conjugated magnetic nanoparticle	20 nm	Targets human breast cancer and is conjugated with radioactive indium	[24]
Antibody or aptamer-conjugated magnetic nanoparticle	10 nm	Targets tumor cells and stabilizes the colloidal solution	[25–28]
Magnetic nanoparticle with encapsulated antitumor drug	20–30 nm	Controlled drug release	[29,30]

NPrCAP: N-propionyl-cysteaminylphenol.

Processes of immunogenic cell death by MNHT & hyperthermia-induced HSPs

A new concept of immunogenic cell death has been proposed [32]. The clearance of dying tumor cells by dendritic cells (DCs) and macrophages that engulf, process and present dying tumor cell material to adaptive immune cells is responsible for the subsequent induction of antitumor immune responses (Figure 1). These immunogenic characteristics are mainly mediated by molecules called 'damage-associated molecular patterns' (DAMPs). Many immunogenic factors has been identified as DAMPs: endoplasmic reticulum (ER) protein calreticulin [33], HSPs [34–36], HMGB1 [37], end-stage degradation products (e.g., ATP, DNA, RNA and uric acid) [38–40], S100 proteins [41] and sphingosine [42]. DAMPs are normally retained within healthy cells and are relocated in dying cells; they are exposed on plasma membrane, secreted extracellularly and produced as end-stage degradation products. They interact with pattern-recognition receptors (e.g., RIGI-like receptors, NOD-like receptors and Toll-like receptors) and stimulate the immune system. Other cell surface receptors, such as RAGE, TIM3, P2Y2, P2X7, G2A and S1PR1–5, also involve and modulate the immune response. The diversity of DAMPs is related to the type of cell death, cells and tissue injuries they are involved with [43].

Hyperthermia may induce multiple distinct types of tumor cell death, such as apoptosis, necrosis and autophagy, which may occur concurrently or successively. The immunogenicity of these dying cells with different types is somehow mediated by changes in the composition of the cell surface and the secretion of soluble molecules, allowing the immune effectors to sense immunogenicity. Asin *et al.* also observed that when MNP-loaded DCs were exposed to an AMF, a massive and sudden disruption of the lysosomes containing the MNPs may have triggered the death of those cells and subsequent release of lysosomal content into the extracellular space, which in turn could have resulted in cell death [44]. It remains to be elucidated as to which kinds of cell death are mainly induced by hyperthermia.

Hyperthermia has been shown to modify blood circulation and thereby to deliver oxygen into the tumor tissue, resulting in increased metabolism, which sensitizes tumor cells to radio- and chemo-therapy [45] and modulates the immune system. Recently, Frey *et al.* reviewed hyperthermia-induced modulations of the immune system in WBHT and LMHT [46]. WBHT and LMHT modulate both innate and adaptive immune responses; macrophages, natural killer (NK) cells, granulocytes and T and B cells are modulated in complex fashions by WBHT and LMHT. Importantly, the expression of HSPs in heated cells is induced in hyperthermia treatments. HSPs are abundant intracellular proteins that function as molecular chaperons that control the folding and prevent the aggregation of proteins [47]. Because the expression of HSPs protects cells from heat-induced apoptosis [48], HSP expression has been considered to be a complicating factor that causes thermotolerance in hyperthermia. An advantage of hyperthermia is the feasibility of frequent, repeated treatments [49]. However, conventional hyperthermia protocols have been performed only once or twice per week at an interval of more than 48 h in order to prevent the thermotolerance [50] caused by HSP expression.

Yanase *et al.* [18] and Ito *et al.* [49], however, found that thermotolerance is overcome in MNHT and repeated hyperthermia treatment at an interval of 24 h, which is very effective for the complete regression of tumor tissues. The application of MCLs led to the formation of stable deposits in tumor tissues, thus allowing repeated hyperthermia treatment without repeated injections of MCLs. These authors also reported that repeated hyperthermia treatment could primarily cause tumor necrosis [51], and that a large amount of HSP70 was induced in the tumor tissue. Their findings appear to correlate well with the report of Basu *et al.*, which indicated that necrotic but not apoptotic cell death leads to the release of HSP70, HSP90, gp96 and calreticulin [52]. Their study also suggested that HSPs stimulate macrophages to secrete cytokines and augment the expression of MHC class I antigens and costimulatory molecules on DCs [52].

Recent reports have demonstrated the importance of HSPs in immune reactions, including HSP70, HSP90 and gp96, and suggested that HSPs chaperon tumor antigens [53–56]. With regards to the mechanism of antitumor immunity induced by hyperthermia, one key component is the heat-induced augmentation of tumor immunogenicity due to the presentation of antigenic peptides via the MHC class I antigens of tumor cells (top of Figure 2). Srivastava *et al.* proposed a 'relay line model' for tumor antigenic peptide transfer during antigen processing and presentation by HSPs [53], involving the following steps: tumor antigens are decomposed to peptides within the proteasome in tumor cells; the peptides are bound to HSP70 or HSP90, carrying these peptides to the ER via the transporter associated with antigen processing; the peptides are then transferred to gp96 in the lumen of the ER; in the terminal step, gp96 transfers the peptides to the MHC class I–β_2 microglobulin complexes.

Based on the findings on the functional role of HSPs in potentiating tumor antigen-specific immune responses, clinical trials have been implemented. A clinical study of an autologous tumor-derived gp96–peptide complex vaccine (HSPPC-96, Oncophage®; Antigenics, Inc.,

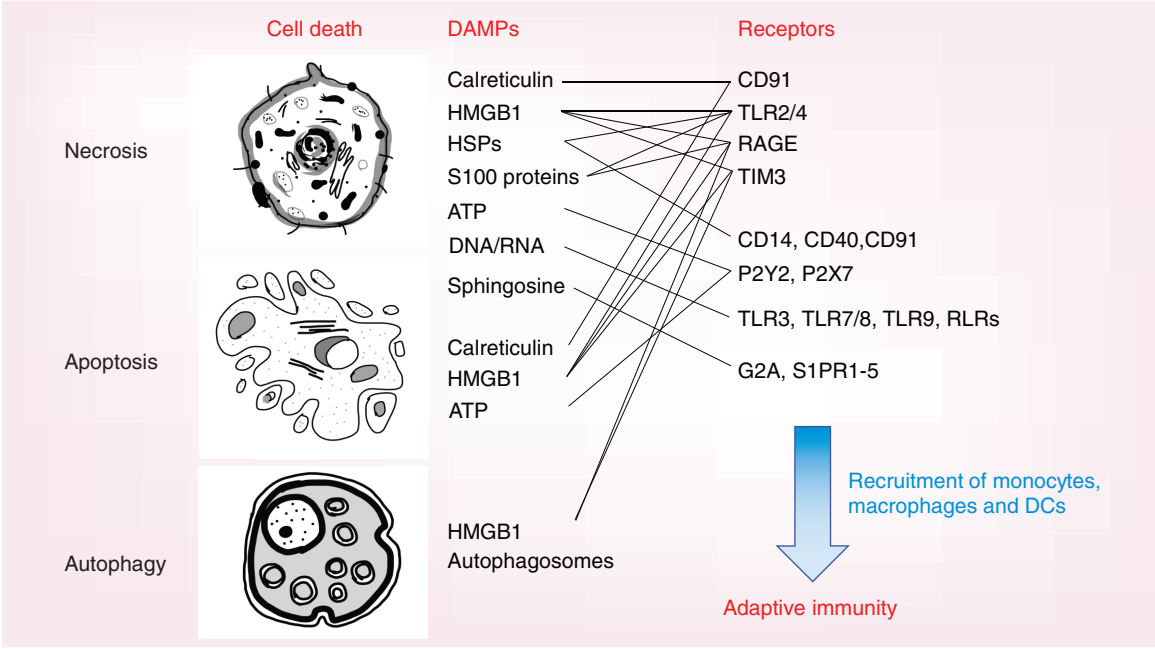

Figure 1. Immunogenic cell death. Damage-associated molecular patterns released from various forms of dying cells recruit immune cells and induce antitumor immunity.

MA, USA) was performed in 2002; the feasibility of its use in treating metastatic melanoma patients has been demonstrated [57,58]. Udono and Srivastava reported that the dose of HSP70–peptide complexes is directly associated with the vaccination effect [59]. Surgery is required in the case of HSPPC-96, because HSP–peptide complexes must be extracted from autologous tumors in order to prepare the vaccine. By contrast, no surgery or extraction is necessary for MNHT treatment. The HSPs in the tumor can be regarded as an antigen source, and 1 g (~10^8 cells) of tumor tissue may contain approximately 2 mg of HSP70. Furthermore, the expression of HSP70 was enhanced and tissue lysis via necrosis was observed throughout the tumor in the MNHT system [60–62]. This represents a much higher dose of HSP–peptide complexes being utilized in MNHT than that used for the clinical trials of HSPPC-96. Therefore, the MNHT protocol should be optimized so that larger amounts of HSP–peptide complexes (possibly including HSPs such as HSP90 and gp96) are expressed *in situ*, and the vaccination effect resulting from HSP–peptide complexes becomes effective not only for the local MNHT-treated tumor, but also for distant MNHT-untreated tumors. Similar results have been reported by Calderwood *et al.* [63] and Graner *et al.* [64].

Mechanism of antitumor immunity induced by MNHT

Yanase *et al.* observed antitumor immune responses induced by hyperthermia using MCLs in an experimental T-9 rat glioma model in which a tumor was transplanted into each femur of rats [8]. When only one tumor with MCL injection was heated above 43°C by MNHT, the other tumor also disappeared completely with no increase of the temperature in the tumor without MCL injection. The rats that had been cured by MNHT were rechallenged with T-9 cells 3 months later, and all tumors disappeared after a period of transient growth. Immunocytochemical assays revealed that the immune response was mediated by both $CD8^+$ and $CD4^+$ T cells and accompanied by a marked augmentation of tumor-selective cytotoxic T-lymphocyte (CTL) activity. The induction of systemic antitumor immune responses by MNHT after AMF exposure has also been reported recently in several B16 mouse melanoma models using MNPs conjugated with NPrCAP [21–23]. Their MNHT treatment caused cytotoxic as well as heat-shock responses, which led to the elicitation of antitumor immune responses as examined by tumor rechallenge rejection and CTL induction. These results may indicate that the administration of therapeutic MNPs is an effective approach for further development of novel antitumor hyperthermia treatments, in as much as selective, strong antitumor effects are induced by both the systemic cell-mediated immune responses and the local *in situ* heat-mediated cell death.

Sato *et al.* proposed a tumor antigen-recognition mechanism similar to that of cytotoxic and heat-shock responses [60]. They used MNPs conjugated with NPrCAP and applied AMF in a mouse melanoma model. The hyperthermia treatment caused cytotoxic as well as heat-shock responses, which induced antitumor

immune responses. The level of HSP70 was increased in the cell lysate after MNHT. Melanoma-specific CD8+ T-cell responses to DCs loaded with hyperthermia-treated tumor lysates were enhanced when compared with nontreated tumor lysates. When HSP70 was immunodeleted from hyperthermia-treated tumor cell lysates, specific CD8+ T-cell responses were abolished. From these results, the authors concluded that the antitumor immune response induced by MNHT is derived from the release of HSP–peptide complexes from degraded tumor cells.

The antitumor activity of MNHT may therefore consist of two major arms: direct killing by hyperthermia and immune-mediated antitumor effects. HSP-mediated antitumor immunity may be caused by a vaccine-like effect of HSP–peptide complexes released from dying tumor cells. A proposed scenario [61,62] in which HSPs function during successive stages of an antitumor response after MNHT is summarized in Figure 2, and outlined below:

Impaired tumor immune responses could be due to poorly immunogenic tumor cells with low concentrations of intracellular HSP–peptide complexes, insufficient function of the endogenous antigen-processing machinery and low levels of MHC class I–peptide complexes at their cell surfaces.

Sublethal stress responses induced by MNHT result in increased levels of intracellular HSP–peptide complexes, enhanced function of the endogenous antigen-processing machinery and increased density of MHC class I–peptide complexes at cell surfaces. These tumor cells are then recognized directly by MHC class I-restricted CTLs. Tumor tissue is heated to a minimum temperature of above 43°C in MNHT, which leads to an increase in the total amount of released HSPs compared with that from temperatures above 38–40°C by WBHT and LMHT.

Dying tumor cells, which are killed by lethal MNHT treatment, release their intracellular contents, including HSP–peptide complexes.

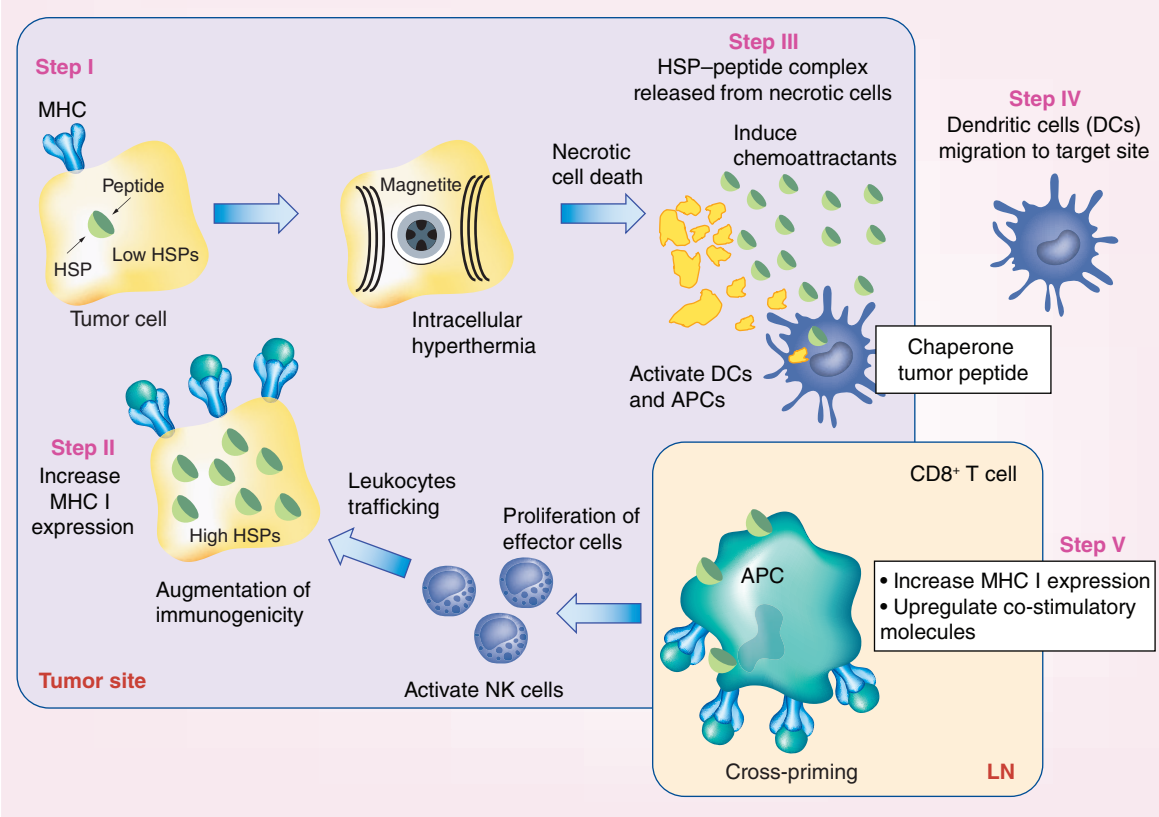

Figure 2. Mechanism of induction of anti-tumor immune response by magnetic nanoparticle-mediated hyperthermia. **(Upper left)** A poorly immunogenic tumor cell with a low concentration of intracellular HSP-peptide complexes. **(Lower left)** Increased levels of intracellular HSP–peptide complexes, enhanced processing of endogenous antigens and an increase in the density of MHC class I–peptide complexes. **(Upper middle)** Dying tumor cells release their intracellular contents, including HSP–peptide complexes. **(Upper right)** DCs activate neighboring monocytes in order to produce proinflammatory cytokines and recruit APCs. **(Lower right)** The HSP–peptide complexes are taken up by DCs and are in turn presented to T cells via MHC class I (cross-priming) and/or II antigens.
APC: Antigen-presenting cell; DC: Denritic cell; HSP: Heat-shock protein.

The production of proinflammatory cytokines and the recruitment of antigen-presenting cells (APCs) from neighboring cells are induced by the release of HSPs and/or antigenic peptides from the dying tumor cells. HSP70 directly activates APCs, stimulates monocytes to secrete cytokines and induces DC maturation via CD14 and/or CD91 receptors [65]. Therefore, HSP70 is considered to be a natural adjuvant [56]. This cytokine-like ability of HSP70 in terms of stimulating the innate immune system is independent of the peptides that HSP70 chaperones [66].

The released HSP–peptide complexes are taken up by APCs that express receptors such as CD91 and CD40 [67,68]. The interaction of HSP–peptide complexes with these receptors leads to receptor-mediated endocytosis, processing of the antigenic peptide by the endogenous MHC class I (cross-priming) and/or classxII pathways and re-presentation on the cell surface to T cells.

Tumor-specific CTLs induced by cross-priming are expected to infiltrate into the tumor and exert antitumor effector activity against the tumor cells, which increases their immunogenicity by MNHT.

Combination effects of MNHT & immunotherapy

In a tumor-bearing host, the aforementioned steps appear to be hampered by immunosuppressive cells and the microenvironment. Therefore, in order to maximize the effects of MNHT and to generate a stronger antitumor immune response, the combination of MNHT and immune induction should be further developed. Successful approaches using this strategy have been demonstrated in several MNHT experiments [69–71]. GM-CSF plays an important role in the activation of APCs [72]. DCs are the most potent APCs and they can induce an immune response to tumors via antigen uptake and maturation [73]. In order to improve antigen presentation and cross-presentation, injections of GM-CSF [69] or immature DCs [70,71] were combined with MNHT, which showed significant results. IL-2 is a potent stimulator of lymphocyte proliferation and augments the activity of CTLs [74]. The combination of the injection of IL-2 and MNHT demonstrated significantly improved results [69].

The modulation of NK cells in hyperthermia treatment, however, appears to be contradictory. NK cell activity was found to be decreased at a higher temperature [75], while it was observed to be enhanced by WBHT *in vivo* [76]. T and B cells are also modulated by heat in complex fashions [46,77]. The cell death rate is dramatically increased at higher temperatures [3]. However, further experiments are necessary in order to optimize the effects of hyperthermia and immunotherapy in order to improve and provide a stronger antitumor immune response.

Clinical trials of MNHT treatment

Many clinical trials of LMHT or WBMT treatment combined with chemotherapy or radiotherapy have been conducted, and some of them were reviewed by Van der Zee [2]. They can be accessed from the websites of ClinicalTrials.gov for US trials, the EU Clinical Trials Register for European trials and the International Clinical Trials Registry Platform for international trails. In the case of MNHT, several clinical trials have been reported.

Gneveckow *et al.* developed the clinical 100-kHz magnetic field applicator MFH® 300F (MagForce Nanotechnologies AG, Berlin, Germany) with a magnetic field strength of up to 18 kA/m in a cylindrical treatment area with a 20-cm diameter and aperture height of up to 300 mm [78]. In 2005, Johannsen *et al.* published the first clinical application of MNHT treatment in recurrent prostate cancer using the MFH 300F applicator [79]. In this study, the feasibility of hyperthermia was evaluated using the MFH 300F applicator and aminosilane-coated magnetite nanoparticles. The MNPs were administered via transperitoneal injection into the prostate of a 67-year-old patient under transrectal ultrasound and fluoroscopy guidance. In this first treated patient, the maximum and minimum intraprostatic temperatures were at 48.5 and 40.0°C, respectively, during the first treatment, and at 42.5 and 39.4°C, respectively, during the sixth treatment. Later, they reported more detailed clinical results for prostate cancer [80,81] and glioblastoma [82–84]. However, detailed immunological characteristics of the patients have not been reported.

Matsumine *et al.* reported a novel MNHT system for patients with bone metastases in their extremities [85]. They used a calcium phosphate cement containing MNPs. Calcium phosphate cement is an injectable biocompatible bone substitute and has been used to fill bone defects following the resection of bone tumors. AMF was applied postoperatively from days 8–29 (to a total of ten AMP exposures). This MNHT treatment has been performed for 23 patients with 25 metastatic bone lesions. The radiographic outcome was assessed according to the following criteria: 'Excellent' indicating 'reduced with visible bone formation', 'Good' meaning 'not progressive for more than 3 months' and 'Poor' meaning 'progressive'. As a result, eight lesions (32%) had 'Excellent' outcomes, while 16 lesions (64%) and one lesion (4%) showed 'Good' and 'Poor' outcomes, respectively.

Preliminary results from a MHNT Phase I clinical trial were reported recently [23]. This study uti-

lized MNPs conjugated with NPrCAP, which is the substrate of the melanin-forming enzyme tyrosinase in melanoma cells. The therapeutic protocol of this human clinical trial essentially followed the outline and results of previous experimental animal studies [86]. Among four stage III and IV melanoma patients to whom this treatment was applied, two showed significant clinical improvement, with marked regression of distant skin metastases in one stage IV patient who survived for 30 months and with regression of local lymph node metastases in one stage III patient who survived for more than 32 months. The original tumor was excised after three local injections of MNP–NPrCAP conjugates in order to examine the local immune responses where the MNHT treatment was carried out. Dense aggregates of lymphocytes and macrophages were seen within and around the necrotic melanoma tissues. Interestingly, these lymphocytes revealed almost identical distributions of $CD8^+$ T cells and MHC class I cells.

Imai *et al.* conducted a Phase I clinical trial of MNHT treatment using MCLs [87]. Patients with head-and-neck, breast or soft-tissue malignant tumors that were refractory to conventional therapy were enrolled. Following the direct injection of MCLs into the tumors, AMF was applied to the tumor for 30 min twice at an interval of 24 h, and the output power of AMF was adjusted so that the temperature of the skin overlying the tumor was elevated to approximately 43°C. Two weeks after the administration of MCLs, the treated tumor was excised under local anesthesia and the effect of MNHT treatment was examined pathologically. The results of this clinical trial are summarized in Table 2. In all six patients, increased temperatures up to 43°C were observed at the injection site. No significant adverse effects of using MCLs were observed in this clinical trial. In the pathological sections, necrotic changes in more than a third of the total tumor cells were observed in four out of six cases.

Conclusion

Significant efforts have been made to construct a safe and effective MNHT system. MNHT effectively heated local tumor tissue above 43°C without damaging surrounding normal tissues, and thereby the tumor volume decreased markedly and significant tumor regression was observed in animal models. MNHT application results in the expression of a significant level of HSPs within and around the tumor tissue and causes strong tumor-specific immune responses. Many *in vivo* experimental results have suggested that MNHT systems confer antitumor immunity via the release of HSP70–peptide complexes during tumor cell death *in vivo*, and a scenario for antitumor immune induction has been presented. Several clinical trials have been started, and the time that MNHT provides an effective and novel approach to antitumor therapy may be expected to come soon.

Future perspective

Achieving a local heating to tumor tissue above 43°C without damaging surrounding normal tissues is the fundamental characteristic of MNHT. The technique consists of accumulating MNPs into tumor tissue followed by exposure to AMF. With regards to MNPs, however, almost all MNPs in the past MNHT treatments were injected directly into the tumor tissue. Attempts have been made recently to attach tumor-

Table 2. Summary of a Phase I clinical trial at Nagoya University Hospital, Japan.

Cancer type	Age (years)/sex	Site	Pathological effect	Adverse effects
Papillary thyroid	79/female	Anterior neck	1a	None
Breast	52/female	Right side chest	2	None
Papillary thyroid	45/female	Left neck	0	None
Tongue	34/male	Left neck	0	None
Soft-tissue sarcoma	32/female	Right sole Right knee occipital	1b 1a	None
Follicular thyroid	55/male	Right neck (center of the target) Right neck (pheripheal zone of the target)	3 1a	None

Pathological effect: 0 = ineffective; 1a = minor effect; 1b = moderate effect; 2 = considerable effect; 3 = complete effect.

specific tags to MNPs, such as mAbs [24–28] or a tumor-specific transporter, NPrCAP [21–23]. Given systemically, such nanoparticles can reach and bind specifically to the tumor tissue, and relatively uniform local heating of the tumor tissue above 43°C will be feasible in the near future from the beginning of MNHT treatment. If MNPs with tumor-specific tags are developed, they could be applied for both MRI tumor diagnosis (molecular imaging) and MNHT. The concentration required for MNHT is several orders of magnitude higher than that necessary for MRI tumor diagnosis. The size, shape and magnetic and surface properties of MNPs should therefore be optimized for this purpose. The development of AMF applicators is also an important issue for applying MNHT to various forms of cancers with different sizes, shapes and locations. While significant progress has been made [23,78,85,87], more sophisticated applicators may still need to be developed. In addition, the development of noninvasive thermometry that accurately measures or estimates the temperature distribution *in situ* within and outside tumor tissues during MNHT treatment also needs to be developed, as reviewed by Kaddi *et al.* [88] and Rodrigues *et al.* [89].

In vivo experimental studies have indicated that MNHT can induce the regression of not only local tumor tissue exposed to heat, but also distant metastatic tumors unexposed to heat [8]. A significant level of HSPs was found to be expressed within and around the tumor tissue, causing strong tumor-specific immune responses. Many *in vivo* experiments indicated that MNHT systems confer antitumor immunity via the release of HSP70–peptide complexes during tumor cell death, and a scenario for antitumor immune induction has been presented in this article. Induction of immune responses by hyperthermia will be the essential determinant of its therapeutic outcome in terms of a long-term effect rather than a short-term, direct cytotoxic effect on tumor cells, but it remains to be elucidated as to which kinds of cell death are primarily induced. It is hoped that MNHT will provide a novel approach to establishing the basic core modalities for the further development of antitumor thermoimmunotherapy.

MNHT combined with chemotherapy, radiotherapy or immunotherapy will improve the therapeutic outcome of malignant tumor patients. MNHT may also be applied for the development of additional therapeutic approaches besides simple antitumor thermotherapy. For example, Williams *et al.* reported that MNHT may have significant potential for enhancing CTL function and acting as an adjunctive therapy in the eradication of latently infected HIV-positive cells [90]. Van Herwijnen *et al.* reported that HSPs can be the targets of regulatory T cells for therapeutic intervention in rheumatoid arthritis [91].

Acknowledgements

The authors acknowledge Y Tamura (Sapporo Medical University) and A Ito (Kyushu University) for valuable comments regarding the manuscript.

Executive summary

Magnetic nanoparticles suitable for magnetic nanoparticle-mediated hyperthermia

- Magnetic nanoparticle-mediated hyperthermia (MNHT) consists of two major, stepwise technical approaches: the first is to accumulate magnetic nanoparticles (MNPs) into the tumor tissue, and the second is to apply an alternating magnetic field in order to generate local heat through nanoparticles within the tumor tissue.
- Almost all of the MNPs developed in the past are injected directly into tumor tissues. However, several forms of MNPs with tumor-specific tags have been given via systemic administration and found to bind selectively to tumor tissues.

Processes of immunogenic cell death by MNHT & hyperthermia-induced heat-shock proteins

- *In vivo* experimental studies have indicated that MNHT can induce the regression of not only local tumor tissue exposed to heat, but also distant metastatic tumors unexposed to heat.
- Immunogenic cell death induced by hyperthermia contributes to therapeutic outcomes in terms of a long-term antitumor effect rather than a short-term, direct cytotoxic effect on tumor cells.
- MNHT enhances the expression of HSP70, which chaperons tumor antigens and increases immunogenicity during hyperthermia treatment.

Mechanism of antitumor immunity induced by MNHT

- The antitumor activity of MNHT may consist of two major arms: a direct killing effect by hyperthermia and an indirect, immune-mediated antitumor effect.
- The antitumor immune response induced by MNHT derives from the release of heat-shock protein–peptide complexes from the degraded tumor cells.

Combined effects of MNHT-induced hyperthermia & immunotherapy & future clinical trials

- Several preliminary clinical trials have been recently conducted based upon many *in vivo* animal experiments. However, further efforts should be directed towards optimizing the combined effects of direct heat-mediated cell death and indirect immune-mediated cell death in order to develop a better antitumor approach with MNHT.

Financial & competing interests disclosure

This work was partially supported by Grants-in-Aid for Scientific Research (no. 24591897) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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